EXTENDED REPORT

Calprotectin (a major leucocyte protein) is strongly and independently correlated with joint inflammation and damage in rheumatoid arthritis

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Objective: Calprotectin is a major leucocyte protein, shown to correlate well with laboratory and clinical assessments in several inflammatory rheumatic diseases, and large concentrations of calprotectin have been found in synovial fluid from patients with rheumatoid arthritis (RA). The objective of the present study was to examine correlations between calprotectin and joint damage.

Methods: 145 patients with RA were analysed cross sectionally with laboratory (calprotectin, C reactive protein (CRP), and erythrocyte sedimentation rate (ESR)), clinical (28 joint counts (tender, swollen), physician global VAS, DAS28 and RA Articular Damage score (RAAD)), and radiographic (plain hand radiographs; modified Sharp's method) measurements, on the same day.

Results: Calprotectin showed a highly significant correlation with measures of joint damage; modified Sharp score $r = 0.43$ ($p < 0.001$) and RAAD $r = 0.40$ ($p < 0.001$). The association with modified Sharp score and RAAD score was maintained after adjustment for CRP, ESR, rheumatoid factor, DAS28, sex, and age in a multiple regression analysis ($p = 0.018$ and $p = 0.04$, respectively), while neither CRP nor ESR showed any independent associations. Highly significant correlations ($p < 0.001$) were also found between calprotectin and both laboratory and clinical markers of inflammation.

Conclusion: Calprotectin was found to significantly and independently explain the variation in the radiological and clinical assessments of joint damage. Longitudinal studies are required to examine whether calprotectin may predict the progression of joint damage in RA.

During the past two decades there has been increasing interest in calprotectin, a major leucocyte protein, which constitutes about 40–60% of the soluble cytosolic protein content in neutrophilic granulocytes, as well as being a major monocyte/macrophage protein.[1–3] The protein is released during cell activation and turnover.[4 5] Calprotectin is one of the calcium binding proinflammatory S100 proteins—S100A8/S100A9,[6] and it is also called protein MRP-8/MRP-14,[7] calgranulin A/calgranulin B,[8] cystic fibrosis antigen,[9] and L1.[10] The protein is released during the interaction of monocytes with inflammatory activated endothelium, probably at sites of local inflammation,[11] and it binds to endothelial cells and modulates transendothelial migration of leucocytes.[12–14] The protein has been described in synovial tissue in rheumatoid arthritis (RA) patients, where it was found in the lining layer adjacent to the cartilage-pannus junction, which is the primary site of cartilage destruction and bone erosion in arthritis.[15] High calprotectin concentrations have been found in synovial fluid from RA patients, while low concentrations were found in osteoarthritic patients.[16 17] In addition, a highly significant correlation was found between the calprotectin levels in plasma and synovial fluid.[11 16] Calprotectin has a molecular weight of only 36.5 kDa,[18] and may thus diffuse from inflamed joints into the circulation, where it can be measured in plasma.[1 16 19] The half life of calprotectin in plasma is only 5 hours,[20] while the half life in synovial fluid is not known. The acute phase proteins are mainly of hepatic origin, while calprotectin is released locally at the site of inflammation. Calprotectin has been studied in several inflammatory joint diseases where highly significant correlations were found between calprotectin and clinical assessments of disease activity as well as with C reactive protein (CRP) and erythrocyte sedimentation rate (ESR).[21–25] A few studies have also been performed on calprotectin during medical treatment of arthritis, where decreased calprotectin levels were found during clinical response,[11 14 22 26] and in patients with systemic lupus erythematosus, significantly higher levels were found in patients with arthritis.[27]

Since calprotectin is released from activated granulocytes and monocytes/macrophages in arthritic joints, the plasma concentration of this protein may reflect the amount of local inflammation and thus be related to joint damage in RA. In the present study we analysed the associations between the calprotectin levels and the radiographic, clinical, and laboratory assessments of joint damage and inflammation in RA patients.

PATIENTS AND METHODS

Patients

A total of 145 patients (110 females) took part in a 10 year follow-up examination of the Euridiss early RA cohort[28 29] and underwent a comprehensive one day examination including laboratory, clinical, and radiographic assessments. The patients gave written consent according to the Declaration of Helsinki, and the study was approved by the local ethics committee. All patients fulfilled the American Rheumatism Association 1987 revised criteria.[30] Mean (SD) age was 59.9 (12.7) years and the disease duration was 12.7 (1.1) years. Their current drug therapy was: disease modifying antirheumatic drugs (DMARDs), n = 69 (48%); prednisolone, n = 52 (36%) and

Abbreviations: anti-CCP, anti-cyclic citrullinated peptide; CRP, C reactive protein; DMARDs, disease modifying antirheumatic drugs; ESR, erythrocyte sedimentation rate; JRA, juvenile rheumatoid arthritis; NSAIDs, non-steroidal anti-inflammatory drugs; RA, rheumatoid arthritis; RAAD score, RA Articular Damage score; VAS, visual analogue scale

non-steroidal anti-inflammatory drugs (NSAIDs) including coxibs, n = 65 (45%). A total of 96 patients (67%) were classified as rheumatoid factor positive based on previous analyses for IgM-RF applying an ELISA technique.[31]

Laboratory examinations

EDTA blood was centrifuged and the upper third of plasma was frozen at –70°C until all samples were analysed for calprotectin at the same time by use of ELISA,[16 19 32] where normal levels are under 0.9 mg/l. In brief, this ELISA method is performed as follows: 100 µl of standards or samples are diluted 1:50 in an assay buffer containing 10 g/l bovine serum albumin, 50 mmol/l TRIS, 150 mmol/l sodium chloride, 0,5 mmol/l magnesium chloride, 2,5 mmol/l potassium chloride, 0.25 mmol thimerosal, and 0.05% Tween-20, pH 8.0 and added to microtitre wells and shaken for 30 minutes at an ambient temperature. After washing, alkaline phosphatase conjugate is added, and the plates are shaken again. After a final wash, substrate is added, and the optical density is read. The coefficients of variation have been found to be 5% within and 13% between assays.[32] CRP and ESR were measured on the day of the clinical examination with routine standards of the hospital laboratory (CRP measured by use of turbidimetry, with 1 mg/l as the lowest detectable value, and ESR by use of Westergren's method).

Clinical assessments

One rheumatologist (SØ) performed the clinical assessments including the 28 swollen and tender joint counts (28-SJC and 28-TJC) and the physician global assessment on a 100 mm visual analogue scale (VAS). The Disease Activity Score based on the 28 joint counts (DAS28) was calculated. The Rheumatoid Arthritis Articular Damage score (RAAD) was used for a clinical assessment of joint damage, and the method is based on an evaluation of joint damage of 35 large and small joints (score range 0–70). The RAAD score has been shown to be a feasible method for measuring the long term articular damage in large RA populations.[33]

Radiographic examinations

All patients were examined with hand radiographs. Radiographic damage was scored according to van der Heijde's modification of Sharp's method[34 35] under the supervision of two of the co-authors (DvdH or RL) blinded for clinical data. The total score (sum of erosions and joint space narrowing) for hands (range 0–280) was used in the analyses.

Statistical methods

The statistical analyses were performed using SAS 9.1.3 (SAS Institute Inc, Cary, NC, USA) and SPSS software (SPSS Inc, Chicago, IL, USA). The data were not normally distributed, and non-parametric tests were used for bivariate associations (Spearman's rank correlation test). The patients were divided into quartiles according to the calprotectin concentrations and the quartiles were compared with regard to joint inflammation and joint damage using ANOVA. The Mann-Whitney test was performed to analyse for differences between groups. Linear multiple regression analyses were performed with the modified Sharp score and RAAD score as dependent variables, and the raw data of calprotectin, CRP, ESR, rheumatoid factor, DAS28, sex, and age as independent variables. The assumptions of the model were tested using Cook's d, studentised residuals and dfbetas. The levels of significance were set at 0.05.

RESULTS

The median (range) levels of the laboratory markers were: calprotectin 1.8 (0.3–8.7) mg/l, CRP 4 (1–49) mg/l, and ESR 15 (2–63) mm in the first hour. The clinical and radiographic measures had the following median (range) values: DAS28 4.2 (0.8–7.1), 28-SJC 4 (0–22), physician global visual analogue scale (VAS) 21 (0–70), the modified Sharp score 23 (0–150) and RAAD score 5 (0–36).

The plasma concentrations of calprotectin correlated significantly with the laboratory, clinical, and radiographic assessments ($p<0.001$) (table 1). The patients were divided into quartiles depending on the calprotectin concentrations: first quartile 0.3–0.8 mg/l (n = 36), second quartile 0.9–1.7 mg/l (n = 36), third quartile 1.8–3.2 mg/l (n = 36), and fourth quartile 3.3–8.7 mg/l (n = 37). Significant differences ($p<0.001$) were found between the quartiles when analysed for the levels of the laboratory (CRP and ESR) and clinical examinations (DAS28, 28-SJC, and physical global VAS). In addition, the calprotectin quartiles showed significant differences ($p<0.001$) when analysed for the levels of the modified Sharp score as well as the RAAD score (fig 1). When both modified Sharp score and RAAD score were divided into quartiles, the calprotectin levels differed significantly between the quartiles ($p<0.001$).

Patients using prednisolone (n = 52) had significantly higher calprotectin values (mean (SD) 2.9 (1.8) mg/l) than those not using prednisolone (mean (SD) 1.9 (1.6) mg/l), $p<0.001$. In

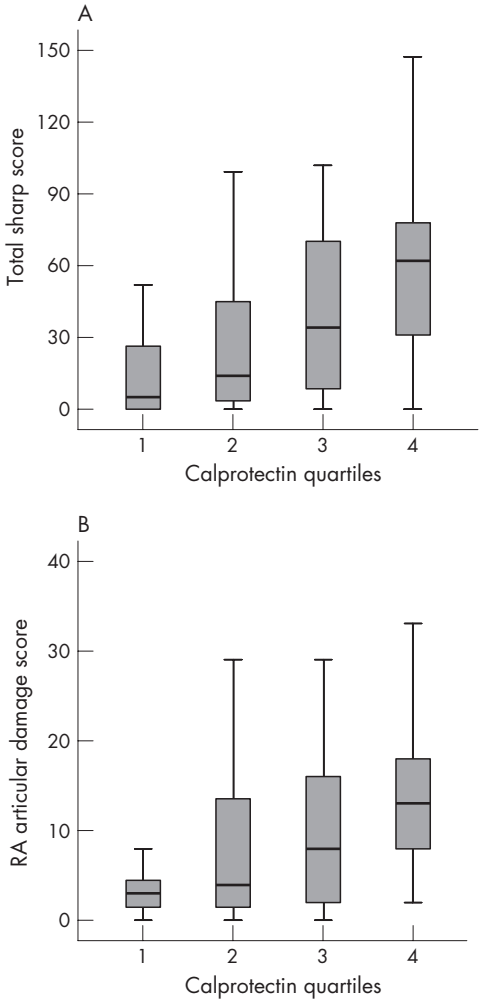

Figure 1 (A) Box plots (median, quartiles, minimum, and maximal values) of the total Sharp score across the quartiles of calprotectin concentrations. There were significant differences between all the quartiles ($p<0.001$). (B) Box plots (median, quartiles, minimum, and maximal values) of the RA articular damage score across the quartiles of calprotectin concentrations. There were significant differences between all the quartiles ($p<0.001$).

Table 1 Spearman's rank correlations between laboratory, clinical, and radiographic assessments of 145 RA patients

	CRP	ESR	DAS28	28 Swollen joint count	Physician global VAS	Modified Sharp score	RAAD score†
Calprotectin	0.57**	0.50**	0.55**	0.49**	0.54**	0.43**	0.40**
CRP		0.55**	0.48**	0.28*	0.46**	0.31**	0.29**
ESR			0.59**	0.26*	0.34**	0.32**	0.30**
DAS28				0.64**	0.65**	0.35**	0.43**
28 Swollen joint count					0.70**	0.53**	0.57**
Physician global VAS						0.62**	0.65**
Modified Sharp score							0.72**

CRP, C reactive protein; ESR, erythrocyte sedimentation rate.
*p<0.01, **p<0.001. †Rheumatoid arthritis articular damage score.

addition, patients using DMARDS (n = 69) had significantly higher levels of calprotectin (mean (SD) 2.8 (1.8) mg/l) than those not using DMARDs (mean (SD) 1.8 (1.4) mg/l), p<0.001. No significant correlation was found between calprotectin and disease duration (r = 0.097, p = 0.25). Significantly higher calprotectin levels (p<0.001) were found in rheumatoid factor positive patients compared with rheumatoid factor negative patients (median (range) calprotectin concentration 2.5 (0.3–8.7) mg/l versus 0.9 (0.3–6.8) mg/l).

In a multivariate, linear regression analyses with the modified Sharp score as the dependent variable, calprotectin, rheumatoid factor, and age were found to be significant covariates, while CRP, ESR, DAS28, and sex were non-significant. When RAAD score was the dependent variable, calprotectin, rheumatoid factor, and DAS28 were significant covariates, while CRP, ESR, sex, and age were non-significant (table 2). After correcting for CRP, ESR, rheumatoid factor, DAS28, sex, and age in the multiple regression analysis, calprotectin remained significantly associated to the modified Sharp score (p = 0.018) and RAAD score (p = 0.04) (table 2). When the analysis was additionally corrected for disease duration, use of DMARDs, or use of prednisolone calprotectin was still significantly associated with the modified Sharp score, p<0.05. With the modified Sharp score as the dependent variable in the linear regression analysis, calprotectin had a R^2 of 0.183, while R^2 was 0.074 for CRP and 0.079 for ESR. When RAAD score was the dependent variable, the R^2 was 0.144 for calprotectin, 0.039 for CRP, and 0.035 for ESR. When calprotectin, CRP, ESR, DAS28, rheumatoid factor, age, and sex were included in the linear regression analysis, the total R^2 was 0.295, while R^2 was 0.221 when a similar analysis was performed with RAAD score as the dependent variable.

DISCUSSION

The main finding in this study was the consistent association between calprotectin and the two measures of joint damage; the modified Sharp score and the RAAD score. Calprotectin, ESR, CRP, and clinical measures of inflammation were all bivariately significantly correlated with the measures of joint damage, but of the inflammatory variables, only calprotectin was independently associated in the multivariate analyses. Radiographic damage is considered as a key end point in clinical studies in RA and has been shown to be associated with long term development of physical disability.[36]

The association between calprotectin and joint damage is biologically plausible. Calprotectin may be released from activated granulocytes and macrophages in the inflamed synovium as well as from the high number of granulocytes in the synovial fluid during inflammation. In synovial fluid from inflamed joints, calprotectin was found to be a major protein when analyses were performed by use of mass spectrometry.[37] This supports the finding of high levels of calprotectin in synovial fluid in several inflammatory joint diseases, where significant correlations were found between the levels in plasma and synovial fluid.[16 17] However, both the modified Sharp score and RAAD score reflect the joint damage as a result of several years of local inflammation. Thus, the present finding of an independent association between calprotectin and measures of joint damage may be explained by a long term elevation of calprotectin in patients with active arthritis.

The calprotectin levels, both in the present and in previous studies,[21 24 25] were found to be associated with the presence of rheumatoid factor, which has been found to be a predictor of longitudinal radiographic progression.[38] However, longitudinal studies should be performed to explore the associations between the levels of rheumatoid factor and/or anti-CCP and calprotectin.

Significantly higher levels of calprotectin were found in patients using prednisolone and/or DMARDs. This may indicate a higher disease activity in these patients, which was supported by the higher levels of the other inflammatory markers as well as the clinical assessments in the treated patients. There are few studies on the influence of medication on calprotectin levels. However, the present study indicates that neither prednisolone

Table 2 Linear multivariate regression analyses with the van der Heijde's modified Sharp score and the Rheumatoid arthritis articular damage (RAAD) score as dependent variables

	Van der Heijde modified Sharp score		RAAD score	
Independent variables	Beta (SE beta)	p Value	Beta (SE beta)	p Value
Calprotectin	5.49 (2.30)	0.018	1.12 (0.55)	0.04
CRP	0.25 (0.39)	0.52	0.002 (0.09)	0.98
ESR	0.08 (0.29)	0.79	−0.08 (0.068)	0.25
DAS28	2.03 (2.56)	0.43	1.6 (0.61)	0.009
Rheumatoid factor	22.79 (6.50)	0.0006	3.33 (1.55)	0.03
Age	0.46 (0.22)	0.039	0.002 (0.053)	0.97
Sex	3.24 (6.53)	0.62	0.95 (1.56)	0.55

CRP, C reactive protein; ESR, erythrocyte sedimentation rate

nor DMARDs per se influence the levels of calprotectin, and this issue ought to be addressed in further studies.

This study is to our knowledge the first to show an independent association between calprotectin levels and joint damage measured by both radiographic and clinical assessments. In a previous study calprotectin was not found to be a significant predictor of joint damage.[39] However, the patients in that study had a broad range of disease duration, indicating a possible ceiling effect for radiographic erosions, and the study had a small number of patients. In future studies, calprotectin should be analysed longitudinally in RA patients without a very destructive disease to find out whether calprotectin is a predictive marker of joint damage.

This study confirmed the findings of previous studies showing that calprotectin is a marker of inflammation, with significant correlations with other laboratory and clinical measures of joint inflammation.[21 22 24 25] High correlations between calprotectin and both laboratory and clinical measures have been found in several inflammatory diseases like juvenile rheumatoid arthritis (JRA),[23 26 40] psoriatic arthritis,[14] reactive arthritis,[41] acute gouty arthritis,[42 43] and systemic lupus erythematosus.[27] Extremely high calprotectin levels were found in patients with flares of systemic JRA.[26 44]

A strength of this study is the use of two separate end points for joint damage. The consistent independent associations between calprotectin and both end points support the robustness of the current finding. Another strength is that all clinical assessments were performed by one investigator, and that the radiographic damage scores were done in a centre with long experience in the assessment of radiographs with the van der Heijde's modified Sharp score. The apparent weakness is the cross sectional design, which does not allow conclusions about the longitudinal predictive value. Previous studies have shown that acute phase reactants, rheumatoid factor, and anti-CCP predict subsequent progression of radiographic damage,[36 45–47] but it has not been possible to develop a clinically useful algorithm for prediction of radiographic progression in early RA.

In conclusion, this study shows that calprotectin is cross sectionally associated with measures of joint damage. Longitudinal studies in RA patients are required to address whether calprotectin may be an independent predictor of subsequent joint damage and whether calprotectin and other markers together may improve the prediction of long term joint damage.

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REFERENCES

1 **Fagerhol MK**, Dale I, Andersson T. A radioimmunoassay for a granulocyte protein as a marker in studies on the turnover of such cells. *Bull Eur Physiopathol Respir* 1980;**16**(Suppl):273–81.
2 **Edgeworth J**, Gorman M, Bennett R, Freemont P, Hogg N. Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells. *J Biol Chem* 1991;**266**:7706–13.
3 **Dale I**, Brandtzaeg P, Fagerhol MK, Scott H. Distribution of a new myelomonocytic antigen (L1) in human peripheral blood leukocytes. *Am J Clin Pathol* 1985;**84**:24–34.
4 **Johne B**, Fagerhol MK, Lyberg T, Prydz H, Brandtzæg P, Naess-Andresen CF, *et al.* Functional and clinical aspects of the myelomonocyte protein calprotectin. *J Clin Pathol: Mol Pathol* 1997;**50**:113–23.
5 **Fagerhol MK**, Dale I, Andersson T. Release and quantitation of a leucocyte derived protein (L1). *Scand J Haematol* 1980;**24**:3913–8.
6 **Foell D**, Roth J. Proinflammatory S100 proteins in arthritis and autoimmune disease. *Arthritis Rheum* 2004;**50**:3762–71.
7 **Odink K**, Cerletti N, Brüggen J, Clerc RG, Tarcsay L, Zwadlo G, *et al.* Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. *Nature* 1987;**330**:80–2.
8 **Wilkinson MM**, Busuttil A, Hayward C, Brock DJH, Dorin JR, van Heyningen V. Expression pattern of two related cystic fibrosis-associated calcium-binding proteins in normal and abnormal tissues. *J Cell Sci* 1988;**91**:221–30.
9 **Dorin JR**, Novak M, Hill RE, Brock DJH, Secher DS, van Heyningen V. A clue to the basic defect in cystic fibrosis from cloning the CF antigen gene. *Nature* 1987;**326**:614–7.
10 **Andersson KB**, Sletten K, Berntzen HB, Dale I, Brandtzaeg P, Jellum E, *et al.* The leucocyte L1 protein: identity with the cystic fibrosis antigen and the calcium-binding MRP-8 and MRP-14 macrophage components. *Scand J Immunol* 1988;**28**:241–5.
11 **Frosch M**, Strey A, Vogl T, Wulffraat NM, Kuis W, Sunderkötter C, *et al.* Myeloid-related proteins 8 and 14 are specifically secreted during interaction of phagocytes and activated endothelium and are useful markers for monitoring disease activity in pauciarticular-onset juvenile rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:628–37.
12 **Kerkhoff C**, Klempt M, Sorg C. Novel insights into structure and function of MRP8 (S100A8) and MRP14 (S100A9). *Biochim Biophys Acta* 1998;**1448**:200–11.
13 **Vogl T**, Ludwig S, Goebeler M, Strey A, Thorey IS, Reichelt R, *et al.* MRP8 and MRP14 control microtubule reorganization during transendothelial migration of phagocytes. *Blood* 2004;**104**:4260–8.
14 **Kane D**, Roth J, Frosch M, Vogl T, Bresnihan B, FitzGerald O. Increased perivascular synovial membrane expression of myeloid-related proteins in psoriatic arthritis. *Arthritis Rheum* 2003;**48**:1676–85.
15 **Youssef P**, Roth J, Frosch M, Costello P, Fitzgerald O, Sorg C, *et al.* Expression of myeloid related proteins (MRP) 8 and 14 and the MRP8/14 heterodimer in rheumatoid arthritis synovial membrane. *J Rheumatol* 1999;**26**:2523–8.
16 **Berntzen HB**, Ölmez Ü, Fagerhol MK, Munthe E. The leukocyte protein L1 in plasma and synovial fluid from patients with rheumatoid arthritis and osteoarthritis. *Scand J Rheumatol* 1991;**20**:74–82.
17 **Uchida T**, Fukawa A, Uchida M, Fujita K, Saito K. Application of a novel protein biochip technology for detection and identification of rheumatoid arthritis biomarkers in synovial fluid. *J Proteome Res* 2002;**1**:495–9.
18 **Dale I**, Fagerhol MK, Naesgaard I. Purification and partial characterization of a highly immunogenic human leukocyte protein, the L1 antigen. *Eur J Biochem* 1983;**134**:1–6.
19 **Røseth AG**, Fagerhol MK, Aadland E, Schjønsby H. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. *Scand J Gastroenterol* 1992;**27**:793–8.
20 **Fagerhol MK**, Nielsen HG, Vetlesen A, Sandvik K, Lyberg T. Increase in plasma calprotectin during long-distance running. *Scand J Clin Lab Invest* 2005;**65**:211–20.
21 **Berntzen HB**, Munthe E, Fagerhol MK. The major leukocyte protein L1 as an indicator of inflammatory joint disease. *Scand J Rheumatol* 1988;(Suppl 76):251–6.
22 **Berntzen HB**, Munthe E, Fagerhol MK. A longitudinal study of the leukocyte protein L1 as an indicator of disease activity in patients with rheumatoid arthritis. *J Rheumatol* 1989;**16**:1416–20.
23 **Berntzen HB**, Fagerhol MK, Østensen M, Mowinckel P, Høyeraal HM. The L1 protein as a new indicator of inflammatory activity in patients with juvenile rheumatoid arthritis. *J Rheumatol* 1991;**18**:133–8.
24 **Brun JG**, Haga H-J, Bøe E, Kallay I, Lekven C, Berntzen HB, *et al.* Calprotectin in patients with rheumatoid arthritis: relation to clinical and laboratory variables of disease activity. *J Rheumatol* 1992;**19**:859–62.
25 **Brun JG**, Jonsson R, Haga H-J. Measurement of plasma calprotectin as an indicator of arthritis and disease activity in patients with inflammatory rheumatic diseases. *J Rheumatol* 1994;**21**:733–8.
26 **Frosch M**, Vogl T, Seeliger S, Wulffraat N, Kuis W, Viemann D, *et al.* Expression of myeloid-related proteins 8 and 14 in systemic-onset juvenile rheumatoid arthritis. *Arthritis Rheum* 2003;**48**:2622–6.
27 **Haga H-J**, Brun JG, Berntzen HB, Cervera R, Khamashta M, Hughes GRV. Calprotectin in patients with systemic lupus erythematosus: relation to clinical and laboratory parameters of disease activity. *Lupus* 1993;**2**:47–50.
28 **Smedstad LM**, Moum T, Guillemin F, Kvien TK, Finch MB, Suurmeijer TPBM, *et al.* Correlates of functional disability in early rheumatoid arthritis: a cross-sectional study of 706 patients in four European countries. *Br J Rheumatol* 1996;**35**:746–51.
29 **Smedstad LM**, Vaglum P, Kvien TK, Moum T. The relationship between self-reported pain and sociodemographic variables, anxiety, and depressive symptoms in rheumatoid arthritis. *J Rheumatol* 1995;**22**:514–20.
30 **Arnett FC**, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.
31 **Van Leeuwen MA**, Westra J, van Riel PLCM, Limburg PC, van Rijswijk MH. IgM, IgA, and IgG rheumatoid factors in early rheumatoid arthritis predictive of radiological progression? *Scand J Rheumatol* 1995;**24**:146–53.
32 **Kristinsson J**, Røseth A, Fagerhol MK, Aadland E, Schjønsby H, Børmer OP, *et al.* Fecal calprotectin concentration in patients with colorectal carcinoma. *Dis Colon Rectum* 1998;**41**:316–21.
33 **Zijlstra TR**, Bernelot Moens HJ, Bukhari MAS. The rheumatoid arthritis articular damage score: first steps in developing a clinical index of long term damage in RA. *Ann Rheum Dis* 2002;**61**:20–3.

34 **Van der Heijde DM**, van Riel PL, Nuver-Zwart IH, Gribnau FW, van de Putte LB. Effects of hydroxychloroquine and sulphasalazine on progression of joint damage in rheumatoid arthritis. *Lancet*, 1989;i, 1036–8.
35 **Van der Heijde D**. How to read radiographs according to the Sharp/van der Heijde Method. *J Rheumatol* 2000;**27**:261–3.
36 **Ødegård S**, Landewé, van der Heijde D, Kvien TK, Mowinckel P, Uhlig T. Association of early radiographic damage with impaired physical function in rheumatoid arthritis. A ten-year, longitudinal observational study in 238 patients. *Arthritis Rheum* 2006;**54**:68–75.
37 **Liao H**, Wu J, Kuhn E, Chin W, Chang B, Jones MD, *et al*. Use of mass spectrometry to identify protein biomarkers of disease severity in the synovial fluid and serum of patients with rheumatoid arthritis. *Arthritis Rheum* 2004;**50**:3792–803.
38 **Van Zeben D**, Hazes JMW, Zwinderman AH, Cats A, van der Voort EAM, Breedveld FC. Clinical significance of rheumatoid factors in early rheumatoid arthritis: results of a follow up study. *Ann Rheum Dis* 1992;**51**:1029–35.
39 **Madland TM**, Hordvik M, Haga H-J, Jonsson R, Brun JG. Leukocyte protein calprotectin and outcome in rheumatoid arthritis. *Scand J Rheumatol* 2002;**31**:351–4.
40 **Wulffraat NM**, Haas PJ, Frosch M, de Kleer IM, Vogl T, Brinkman DMC, *et al*. Myeloid related protein 8 and 14 secretion reflects phagocyte activation and correlates with disease activity in juvenile idiopathic arthritis treated with autologous stem cell transplantation. *Ann Rheum Dis* 2003;**62**:236–41.
41 **Hammer HB**, Kvien TK, Glennås A. A longitudinal study of calprotectin as an inflammatory marker in patients with reactive arthritis. *Clin Exp Rheumatol* 1995;**13**:59–64.
42 **Ryckman C**, Gilbert C, de Médicis R, Lussier A, Vandal K, Tessier PA. Monosodium urate monohydrate crystals induce the release of the proinflammatory protein S100A8/A9 from neutrophils. *J Leukoc Biol* 2004;**76**:433–40.
43 **Ryckman C**, McColl SR, Vandal K, de Médicis R, Lussier A, Poubelle PE, *et al*. Role of S100A8 and S100A9 in neutrophil recruitment in response to monosodium urate monohydrate crystals in the air-pouch model of acute gouty arthritis. *Arthritis Rheum* 2003;**48**:2310–20.
44 **Schulze zur Wiesch A**, Foell D, Frosch M, Vogl T, Sorg C, Roth J. Myeloid related proteins MRP8/MRP14 may predict disease flares in juvenile idiopathic arthritis. *Clin Exp Rheumatol* 2004;**22**:368–73.
45 **Jansen LMA**, van der Horst-Bruinsma IE, van Schaardenburg D, Bezemer PD, Dijkmans BAC. Predictors of radiographic joint damage in patients with early rheumatoid arthritis. *Ann Rheum Dis* 2001;**60**:924–7.
46 **Kroot E-JJA**, de Jong BAW, van Leeuwen MA, Swinkels H, van den Hoogen FHJ, van't Hof M, *et al*. The prognostic value of anti-cyclic citrullinated peptide antibody in patients with recent-onset rheumatoid arthritis. *Arthritis Rheum* 2000;**43**:1831–5.
47 **Jansen LMA**, van Schaardenburg D, van der Horst-Bruinsma IE, van de Stadt RJ, de Koning MHMT, Dijkmans BAC. The predictive value of anti-cyclic citrullinated peptide antibodies in early arthritis. *J Rheumatol* 2003;**30**:1691–5.